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(54) Title: LIPOSOMAL GEMCITABINE COMPOSITIONS FOR BETTER DRUG DELIVERY

(57) Abstract: The present invention is for novel compositions and methods for treating cancer, particularly, for treating cancer in mammals and more particularly in humans. The therapeutic compositions of the present invention include liposome entrapped gemcitabine in which the liposome can contain any of a variety of neutral or charged liposome-forming compounds including cardiolipin.

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LIPOSOMAL GEMCITABINE COMPOSITIONS FOR BETTER DRUG DELIVERY

FIELD OF THE INVENTION

[0001] This invention pertains to formulations and methods for making and using gemcitabine-containing liposomes.

DESCRIPTION OF THE BACKGROUND

[0002] Gemcitabine is a nucleoside analogue that exhibits antitumor activity. Gemcitabine exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S-phase) and also blocking the progression of cells through the G_1/S-phase boundary. Gemcitabine is metabolized intracellularly by nucleoside kinases to the active diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleosides. The cytotoxic effect of gemcitabine is attributed to a combination of two actions of the diphosphate and the triphosphate nucleosides, which leads to inhibition of DNA synthesis. First, gemcitabine diphosphate inhibits ribonucleotide reductase, which is responsible for catalyzing the reactions that generate the deoxynucleoside triphosphates for DNA synthesis. Inhibition of this enzyme by the diphosphate nucleoside causes a reduction in the concentrations of deoxynucleotides, including dCTP. Second, gemcitabine triphosphate competes with dCTP for incorporation into DNA. The reduction in the intracellular concentration of dCTP (by the action of the diphosphate) enhances the incorporation of gemcitabine triphosphate into DNA (self-potentiation). After the gemcitabine nucleotide is incorporated into DNA, only one additional nucleotide is added to the growing DNA strands. After this addition, there is inhibition of further DNA synthesis. DNA polymerase epsilon is unable to remove the gemcitabine nucleotide and repair the growing DNA strands (masked chain termination). In CEM T lymphoblastoid cells, gemcitabine induces internucleosomal DNA fragmentation, one of the characeristics of programmed cell death.

[0003] The U.S. Food and Drug Administration (FDA) first approved gemcitabine hydrochloride for sale in the United States in 1996 as an injectable formulation under the tradename Gemzar®. The clinical formulation is supplied in a sterile form for intravenous use only. Vials of Gemzar® contain either 200 mg or 1 g of gemcitabine HCl (expressed as free base) formulated with mannitol (200 mg or 1 g, respectively) and sodium acetate (12.5 mg or 62.5 mg, respectively) as a sterile lyophilized powder. Hydrochloric acid and/or sodium hydroxide may have been added for pH adjustment.

[0004] Gemcitabine demonstrates dose-dependent synergistic activity with cisplatin in vitro. No effect of cisplatin on gemcitabine triphosphate accumulation or DNA double-strand breaks was observed. In vivo, gemcitabine showed activity in

combination with cisplatin against the LX-1 and CALU-6 human lung xenografts, but minimal activity was seen with the NCI-H460 or NCI-H520 xenografts. Gemcitabine was synergistic with cisplatin in the Lewis lung murine xenograft. Sequential exposure to gemcitabine 4 hours before cisplatin produced the greatest interaction.

[0005] GEMZAR® is indicated as in combination with cisplatin for the first-line treatment of patients with locally advanced (Stage IIIA or IIIB) or metastatic (Stage IV) NSCLC. GEMZAR® is also available as first-line treatment of the treatment of locally advanced (nonresectable Stage II or Stage III) or metastatic pancreatic cancer (Stage IV) in patients. However, the toxicity of gemcitabine limits the dosage of drug that can be administered to patients. Gemcitabine HCL also has very short half-life in patients. The half-life and volume of distribution depends on age, gender and duration for infusion. Moreover, the development of multidrug resistance in cells exposed to gemcitabine can limit its effectiveness. Consequently, formulations of gemcitabine are needed that sufficiently prolong half-life of gemcitabine and maximize its therapeutic efficacy for example, by minimizing the multidrug resistance of treated cells and limiting its toxicity.

SUMMARY OF THE INVENTION

[0006] The present invention provides for novel gemcitabine compositions, their preparation methods, and their use in treating proliferative diseases such as cancer, particularly in mammals, especially in humans. The compositions of the present invention include liposome-entrapped gemcitabine in which the liposome can contain any of a variety of neutral or charged liposome-forming materials and/or cardiolipin. The liposome-forming materials are amphiphilic molecules such as phosphatidylcholine (PC), cholesterol, phosphatidylglycerol (PG), phosphatidylserine (PS), and the like. The cardiolipin in the liposomes can be derived from natural sources or synthetic. Depending on their composition, the liposomes can carry net negative or positive charges or can be neutral. Preferred liposomes also contain α-tocopherol.

[0007] The term "Gemcitabine" as used herein means Gemcitabine hydrochloride, Gemcitabine free base and Gemcitabine derivatives.

[0008] The liposomal compositions can be used advantageously in conjunction with secondary therapeutic agents other than gemcitabine, including antineoplastic, antifungal, antibiotic among other active agents, particularly cisplatin, antisense oligonucleotides, oxaliplatin, paclitaxel, vinorelbine, epirubicin. The liposomes can be multilamellar vesicles, unilamellar vesicles, or their mixtures as desired. The invention specifically contemplates methods in which a therapeutically effective

amount of the inventive liposomes in a pharmaceutically acceptable excipient are administered to a mammal, such as a human.

[0009] Desirably, the composition and method present one or more of the following advantages: 1) achieve a strong electrostatic interaction between lipids and gemcitabine, 2) avoidance of solubility problems, 3) high gemcitabine and liposome stability, 4) ability to administer gemcitabine as a bolus or short infusion in a high concentration, 5) prolong half-life of gemcitabine, 6) reduced gemcitabine toxicity, 7) increased therapeutic efficacy of gemcitabine, and 8) modulation of multidrug resistance in cancer cells. These and other properties and advantages of the present invention will be apparent upon reading the following detailed description.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0010] In one embodiment, the invention provides a composition including liposomal Gemcitabine and a negatively charged phosholipid (e.g., a first liposome-forming material), and the use of such a composition to treat cellular proliferative diseases. The Gemcitabine in the composition can be Gemcitabine hydrochloride, Gemcitabine free base, one or more Gemcitabine derivatives, or a mixture thereof.

[0011] While the negatively-charged phospholipids can be selected from among a variety of phospholipids having a negative charge, desirably the selection of the negatively charged phospholipids permits the Gemcitabine to become complexed with the negatively-charged phospholipids through electrostatic interaction. One preferred negatively charged phospholipid for inclusion in the formulation is cardiolipin, which can be, for example, natural cardiolipin, synthetic cardiolipin, or a mixture thereof. The cardiolipin can be or comprise a portion of the negatively-charged phospholipid within the composition, and it is desirable for all or a portion of the cardiolipin to be complexed with the Gemcitabine within the composition.

[0012] While the liposomal formulation including the Gemcitabine includes a negatively-charged phosopholipid, the liposomes within the composition can have a net negative or a net positive charge, or they can be neutral. The charge of the liposomes can be influenced, for example, by the presence of other liposome-forming material. In this respect, in addition to the negatively-charged phospholipid (e.g. a cardiolipin), the liposomes can include a second liposome-forming material, for example, one or more lipids such as phosphatidylcholine, cholesterol, α-tocopherol, phosphatidylglycerol and phosphatidyl serine. For example, at neutral pH, positively charged liposomes can be formed from a mixture of phosphatidylcholine, cholesterol and stearyl amine. Alternatively, negatively charged liposomes can be formed from phosphatidylcholine, cholesterol, and phosphatidyl serine.

[0013] The liposomes within the composition can be multilamellar vesicles, unilamellar vesicles, or a mixture thereof. Moreover, the liposomes can be of varying size or substantially uniform in size. For example the liposomes can have a size of about 1 mm or less, and more preferably are in the micron or sub-micron range. For example, the liposomes can have a diameter of about 5 μm or less, such as about 1 μm or less, or even 0.5 μm or less, such as about 0.2 μm or less or even about 0.1 μm or less.

[0014] Generally, the liposomes for use in the present invention can be formed by known techniques. For example, in one preferred technique gemcitabine is dissolved in an organic solvent with negatively charged phospholipids, such as cardiolipin (CL) and other phospholipids as desired and pharmaceutical excipients allowed forming complexes with gemcitabine. The cardiolipin/gemcitabine-containing mixture can be evaporated to form a film in order to facilitate electrostatic interaction and complex formation. Thereafter, solutions containing any additional desired additional lipophilic ingredients can be added to the film and the gemcitabine/lipids complexes dissolved or thoroughly dispersed in the solution. The solution can then be evaporated to form a second lipid film. A polar solvent such as an aqueous solvent can then be added to the lipid film and the resulting mixture vigorously homogenized to produce the present inventive liposomes. In another preferred technique, all of the lipophilic ingredients can be dissolved in a suitable solvent that can then be evaporated to form a lipophilic film. A polar solvent such as an aqueous solvent can then be added to the lipid film and the resulting mixture vigorously homogenized to produce the present inventive liposomes. In yet another alternative method, gemcitabine can be dissolved in a suitable aqueous solvent or buffers. The aqueous of gemcitabine can then be added to the lipid film and the resulting mixture vigorously homogenized to produce liposomes, emulsions and micelles, as desired.

[0015] Where the gemcitabine is dissolved in the lipid film as described above, the dosage form can be conveniently packaged in a single vial to which a suitable aqueous solution can be added to form the liposomes. Alternatively, a two vial system can be prepared in which the lipophilic ingredients are contained as a film in one vial and aqueous ingredients containing gemcitabine are provided in a second vial. The aqueous gemcitabine-containing ingredients can be transferred to the vial containing the lipid film and the liposomes formed by standard methods.

[0016] In a preferred embodiment, the liposomes, once formed, can be filtered through suitable filters to control their size distribution. Suitable filters include those that can be used to obtain the desired size range of liposomes from a filtrate. For example, the liposomes can be formed and thereafter filtered through a 5 micron filter

to obtain liposomes having a diameter of about 5 microns or less. Alternatively, 1 μm, 500 nm, 100 nm or other suitable filters can be used to obtain liposomes of desired size. The present inventive liposomes are stable and can be filtered through microbial retentative filters to have a sterile pharmaceutical product.

[0017] In accordance with the invention gemcitabine is dissolved in a suitable solvent. Suitable solvents are those in which gemcitabine is soluble and which can be evaporated without leaving a pharmaceutically unacceptable residue. For example, non-polar or slightly polar solvents may be used, such as ethanol, methanol, chloroform, methylene chloride or acetone.

[0018] Any suitable negatively charged lipid and cardiolipin preparation can be used in the present invention. For example, cardiolipin can be purified from natural sources or can be chemically synthesized, such as tetramyristylcardiolipin, by such methods as are known in the art. Cardiolipin can be dissolved in a suitable solvent as described above for gemcitabine and the solutions mixed or the cardiolipin can be dissolved directly with gemcitabine.

[0019] In addition to cardiolipin or other negatively-charged phospholipid, any suitable liposome-forming material can be used in the present liposomes. Suitable liposome forming materials include synthetic, semi-synthetic (modified natural) or naturally occurring compounds having a hydrophilic portion and a hydrophobic portion. Such compounds are amphiphilic molecules and can have net positive, negative, or neutral charges. The hydrophobic portion of liposome forming compounds can include one or more nonpolar, aliphatic chains, for example, palmitoyl groups. Examples of suitable liposome-forming compounds include phospholipids, sterols, fatty acids, and the like. Preferred liposome forming compounds include cardiolipin, phosphatidylcholine (PC), cholesterol, phosphatidylglycerol (PG), phosphatidylserine (PS), and α-tocopherol. Phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylinositol (PI), sphingomyelin (SM), ganglioside G_{M1} and polymer modified lipids, such as PEG modified lipids or a combination thereof also can be included.

[0020] As described above for the negatively-charged phospholipids (e.g., cardiolipin) and gemcitabine, the liposome-forming material can be dissolved in a suitable solvent, which can be a low polarity solvent such as chloroform, or a non-polar solvent, such as n-hexane. Other lipophilic ingredients can be admixed with the aforementioned ingredients, the ingredients can then be mixed with gemcitabine and the solvent evaporated to produce a homogeneous lipid film. Solvent evaporation can be by any suitable means that preserves the stability of gemcitabine and other lipophilic ingredients.

[0021] Liposomes can then be formed by adding a polar solution, preferably an aqueous solution, such as a saline solution, to the lipid film and dispersing the film by vigorous mixing. Optionally, the polar solution can contain gemcitabine. The solution can be pure water or it can contain salts, buffers, or other soluble active agents. Any method of mixing can be used provided that the chosen method induces sufficient shearing forces between the lipid film and polar solvent to strongly homogenize the mixture and form liposomes. For example, mixing can be by vortexing, magnetic stirring, and/or sonicating. Multilamellar liposomes can be formed simply by vortexing the solution. Where unilamellar liposomes are desired a sonication or filtration step is included in the process.

[0022] More generally, any suitable method of forming liposomes can be used so long as it provides liposome entrapped gemcitabine. Thus, solvent evaporation methods that do not involve formation of a dry lipid film can be used. For example, liposomes can be prepared by forming an emulsion in an aqueous and organic phase and evaporating the organic solvent. Reverse-phase evaporation, infusion procedures, and detergent dilution, can be used to produce the liposomes. The present invention is intended to encompass liposome-entrapped gemcitabine, without regard to the procedure for making the liposomes.

[0023] The preferred liposome entrapped gemcitabine compositions contains suitable amounts of gemcitabine. Suitable amounts can include from 1 to 50 wt.% gemcitabine, and more preferably 2 to 25 wt.% gemcitabine. Preferred compositions also contain cardiolipin, cholesterol, phosphatidylcholine and α-tocopherol in suitable amounts. The inventive compositions can contain any suitable amount of cardiolipin. Suitable amounts can include from 1 to 50 wt.% cardiolipin, and more preferably 2 to 25 wt.% cardiolipin. The inventive compositions can contain any suitable amount of phosphatidylcholine. Suitable amounts of phosphatidylcholine can include from 1 to 95 wt.% phosphatidylcholine, and more preferably 20 to 75 wt.% phosphatidylcholine. Preferred liposomes of the present invention also contain suitable amounts of α-tocopherol or other suitable antioxidants. Suitable amounts range from 0.001 wt.% to 10 wt.% α-tocopherol, such as, for example, 5 wt.% α-tocopherol. For reference, wt.% refers to the relative mass of each ingredient in the final composition without regard to the amount of added water.

[0024] To improve shelf-life and preserve liposome stability, the present invention provides gemcitabine liposome preparations which can be stored for extended periods of time without substantial leakage from the liposomes of internally encapsulated materials.

[0025] The present invention provides a gemcitabine liposome preparations, which can be dehydrated, stored for extended periods of time while dehydrated, and then rehydrated when and where they are to be used, without losing a substantial portion of loaded gemcitabine during the dehydration, storage and rehydration processes. To achieve these and other objects, the invention, in accordance with one of its aspects, provides gemcitabine liposome preparations which have been dehydrated in the presence of one or more protective sugars. In certain preferred embodiments of the invention, the liposomes are dehydrated with the one or more sugars being present at both the inside and outside surfaces of the liposome membranes. In other preferred embodiments, the sugars are selected from the group consisting of trehalose, maltose, lactose, sucrose, glucose, and dextran, with the most preferred sugars from a performance point of view being trehalose and sucrose. In general, disaccharide sugars have been found to work better than monosaccharide sugars, with the disaccharide sugars trehalose and sucrose being most effective. Other more complicated sugars can also be used. For example, aminoglycosides, including streptomycin and dihydrostreptomycin, have been found to protect liposomes during dehydration.

[0026] The dehydration is preferably achieved under vacuum and can take place either with or without prior freezing of the liposome preparation. The liposomes are preferably dehydrated using standard freeze-drying equipment or equivalent apparatus, that is, they are preferably dehydrated under reduced pressure. If desired, the liposomes and their surrounding medium can be frozen in liquid nitrogen before being dehydrated. Alternatively, the liposomes can also be dehydrated without prior freezing, by simply being placed under reduced pressure.

[0027] It has been found that invented liposomes having a concentration gradient across their membranes can be dehydrated in the presence of one or more sugars, stored in their dehydrated condition, subsequently rehydrated, and the concentration gradient then used to create a transmembrane potential which will load gemcitabine into the liposomes. Alternatively, the concentration gradient can be created after the liposomes have been dehydrated, stored, and rehydrated.

[0028] When the dehydrated liposomes are to be used, rehydration is accomplished by adding diluent, such as water for injection, normal saline, 5% dextrose in normal saline (D5W). The gemcitabine liposomes can be resuspended into the aqueous solution by gentle swirling of the solution. The rehydration can be performed at room temperature or at other temperatures appropriate to the composition of the liposomes and their internal contents.

[0029] The invention includes pharmaceutical preparations that in addition to the liposomal gemcitabine preparation, also include non-toxic, inert pharmaceutically

suitable excipients and processes for the production of these preparations. The invention also includes pharmaceutical preparations in dosage units. This means that the preparations are in the form of individual parts, for example capsules, softgel capsules, pills, suppositories, ampoules and vials, of which the content of liposome entrapped gemcitabine corresponds to a fraction or a multiple of an individual dose. The dosage units can contain, for example, 1, 2, 3 or 4 individual doses or 1/2, 1/3 or 1/4 of an individual dose. An individual dose preferably contains the amount of gemcitabine which is given in one administration and which usually corresponds to a whole, a half or a third or a quarter of a daily dose.

[0030] The abovementioned pharmaceutical preparations are manufactured in the usual manner according to known methods, for example by mixing liposomal gemcitabine with an excipient or excipients. By non-toxic, inert pharmaceutically suitable excipients there are to be understood solid, semi-solid or liquid diluents, fillers, solubilizers, stabilizer and formulation auxiliaries of all kinds.

[0031] The active compound or its pharmaceutical preparations administered locally, orally, parenterally, intraperitoneally and/or rectally, preferably parenterally, especially intravenously. Suitable amounts are therapeutically effective amounts that do not have excessive toxicity, as determined in empirical studies. Accordingly, any pharmaceutical preparation suitable to the desired route of administration, e.g., tablets, dragees, capsules, pills, granules, suppositories, solutions, suspensions and emulsions, pastes, ointments, gels, creams, lotions, powders and sprays, can be used. Suppositories can contain, in addition to the liposome-entrapped gemcitabine, suitable water-soluble or water-insoluble excipients. Suitable excipients are those in which the inventive liposomal entrapped gemcitabine are sufficiently stable to allow for therapeutic use, for example polyethylene glycols, certain fats, and esters or mixtures of these substances. Ointments, pastes, creams and gels can contain suitable excipients in which the liposome-entrapped gemcitabine is stable and can contain additives such as eucalyptus oil and sweeteners like saccharin.

[0032] The present invention also includes the use of the active compound according to the invention and of pharmaceutical preparations which contain the active compound according to the invention in human and veterinary medicine for the prevention, amelioration and/or cure of diseases, in particular those diseases caused by cellular proliferation, such as cancer. The composition can be used to treat cancer in any patient in need of such treatment, which is typically a mammalian patient, such as a cow, horse, pig, dog or cat. For example, dog lymphoma can be treated effectively with the present gemcitabine formulation. However, the present formulation is particularly preferred for use in the treatment of human patients, particularly for cancer

and other diseases caused by cellular proliferation. Examples of cancers treatable by this invention include, but not limited to lung cancer (including, but not limited to unresectable, advanced non small cell lung cancer); breast cancer; testicular cancer; ovarian cancer; gastro intestinal cancers including colon, rectal, pancreatic, and gastric cancers, hepatocellular carcinoma; head and neck cancers; prostate cancer; renal cell carcinoma; adenocarcinoma; sarcomas; lymphomas; leukemias; and mycosis fugoides; melanoma; high grade glioma, glioblastoma and brain cancers.

[0033] The gemcitabine should preferably be present in the abovementioned pharmaceutical preparations in a concentration of about 0.1 to 50, preferably of about 0.5 to 25, percent by weight of the total mixture. Depending, in part, on the route of administration, the usual initial dose of gemcitabine is about 600-1500mg/m^2. In a human, for example, preferably, about 800-1300 mg/m^2 is administered. However, it can be necessary to deviate from the dosages mentioned and in particular to do so as a function of the nature and body weight of the subject to be treated, the nature and the severity of the illness, the nature of the preparation and if the administration of the medicine, and the time or interval over which the administration takes place. Thus it can suffice in some cases to manage with less than the abovementioned amount of active compound while in other cases the abovementioned amount of active compound can be exceeded. However, determining an optimal dosage is within the ordinary skill of a practitioner in this field, and the particular required optimum dosage and the type of administration of the gemcitabine can be determined by one skilled in the art, by available methods.

[0034] One significant advantage of the present composition is that it provides a method of modulating multidrug resistance in cancer cells that are subjected to gemcitabine. In particular, the present liposomal compositions reduce the tendency of cancer cells subjected to chemotherapy with gemcitabine to develop resistance thereto, and reduces the tendency of treated cells of developing resistance to other therapeutic agents, such as cisplatin, vindesine, taxol, 5-fluorouracil (5-FU) or leucovorin, for example. Thus, other agents can be advantageously employed with the present treatment either in the form of a combination active with gemcitabine or by separate administration. Preferred agents other than gemcitabine include antineoplastic, antifungal, and antibiotic among other active agents; particularly cisplatin, antisense oligonucleotides (especially an oligonucleotide antisense to raf (e.g., 5'-GTGCTCCATTGATGC-3' (SEQ ID NO:1)), such as liposomal formulation of anti-c-raf oligonucleotides (see, e.g., U.S. Patents 6,126,965 and 6,559,129), oxaliplatin, paclitaxel, vinorelbine, epirubicin. Another advantage of the present composition is that the present liposomal compositions reduce the irritation, local tissue necrosis,

and/or thrombophlebitis. By using the present liposomal compositions, the extravasation injuries is significantly reduced since the free gemcitabine is not in contact with the tissue directly.

EXAMPLE 1

[0035] This is an example of lipid formulation according to the invention, with gemcitabine hydrochloride.

[0036] Lipids (85-500 μmole) were dissolved in organic solvent. The mixture was stirred gently and the solvents were evaporated under vacuum at 40-60°C to form a thin dry film of lipids. Gemcitabine hydrochloride (70 μmole) was dissolved in 5 ml of 30 mM acetate buffer, pH 3.0. Liposomes were formed by adding the drug solution to the lipid film and aggressively mixing the components by votexing. The liposomes formed then were extruded through two stacked 0.2 μm and 0.1 μm pore size polycarbonate filters to reduce the particle size. The liposome mean diameter was determined using dynamic light scattering (DLS) technique with a Nicomp 380 Submicron Particle Sizer (Particle Sizing Systems, Santa Barbara, CA) equipped with auto dilution function. The gemcitabine binding efficiency in the liposome was determined by centrifuging an aliquot of the subject liposomes at 58,000 rpm for 2 hours at 4°C. Thereafter the drug was analyzed using high pressure liquid chromatography (HPLC). Generally the binding efficiency of gemcitabine in liposomes between 15-80% of the initial input dose.

[0037] Data for several formulations are presented in Table 1, and Figure 1 shows the effect of the molar ratio between DOPG and gemcitabine hydrochloride on the gemcitabine binding efficiency in the liposomes. Gemcitabine binding increased with an increasing molar ratio of DOPG to gemcitabine from 0.5:1 to 5:1. However the drug percent binding reached a plateau once the lipid to drug molar ratio exceeded 5:1.

Table 1

Formulation	Charge ratio (-/+)	Drug binding efficiency(%)	Vesicle size (nm)	Liposome formed
1,1',2,2'-Tetramyristoyl Cardiolipin	1.0	18.7	/	yes
1,1',2,2'-Tetramyristoyl Cardiolipin	2.0	33.9	/	yes
1,1',2,2'-Tetralauroyl Cardiolipin	2.0	20.5	201	yes
DOPG	2.0	46.3	114	yes
DOPG	4.0	59.4	/	yes
DOPG	5.0	74.7	116	yes
DOPG: DPPC 80:20	5.0	70.5	119	yes
DOPG: DSPC 80:20	5.0	72.8	115	yes
DOPG: cholesterol 80:20	5.0	67.4	119	yes
DOPG: cholesterol sulfate 80:20	5.0	63.7	198	yes
DOPG: chol.: cardiolipin 70:20:10	5.1	65.0	116	yes
DOPG: DSPC: cadiolipin 70:20:10	5.1	59.8	107	yes
DOPG: DSPC: DSPG 80:10:10	5.1	63.9	94	yes
DOPG: DSPC: chol. 70:20:10	5.0	70.9	118	yes
DOPG: DSPC: chol. 60:20:20	4.0	54.3	132	yes
DMPG	5.0	34.3	39.8	yes
DMPG: cholesterol 80:20	5.0	33.1	92.3	yes

EXAMPLE 2

[0038] This is an example of lipid formulation according to the invention, with gemcitabine free base.

[0039] Gemcitabine free base (76 μmole) was dissolved in organic solvent containing lipids (150-380 μmole). The mixture was stirred gently and the solvents evaporated under vacuum at 40°C to form a thin dry film of lipids and drug. Liposomes were formed by adding 5 ml of 30 mM acetate buffer, pH 3.0 or 5 ml of 20% sucrose pH adjusted to 8.5 with NaOH and mixing the components by votexing. The liposomes formed then were extruded through two stacked 0.2 μm and 0.1 μm pore size polycarbonate filters to reduce the particle size. The liposome mean diameter was determined using dynamic light scattering (DLS) technique with a Nicomp 380 Submicron Particle Sizer (Particle Sizing Systems, Santa Barbara, CA) equipped with auto dilution function. The gemcitabine binding efficiency in the liposome was determined by centrifuging an aliquot of the subject liposomes at 58,000 rpm for 2 hours at 4°C. Thereafter the drug was analyzed using high pressure liquid

chromatography (HPLC). Generally the binding efficiency of gemcitabine in liposomes was between 20-80% of the initial input dose. Data for several formulations are presented in Table 2.

Table 2

Formulation	Lipid/ drug ratio	Drug dissolved in lipid film	pH of the formulation	Drug binding efficiency(%)	Vesicle size (nm)	Liposome formed
DOPC:Chol.: CL 50:30:20	5:1	yes	7.8	23.5	133	yes
DOPC:Chol.: CL 50:30:20	5:1	yes	3.8	33.6	95	yes
DOPG	2:1	yes	4.0	36.8	104	yes
DOPG	5:1	yes	4.3	75.2	105	yes
DOPG	5:1	no	4.0	48.7	110	yes

[0040] All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

[0041] While this invention has been described with an emphasis upon preferred embodiments, variations of the preferred embodiments can be used, and it is intended that the invention can be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the claims.

WHAT IS CLAIMED IS:

1. A method of treating a cellular proliferative disease, comprising administering to a patient in need of such treatment a pharmaceutical composition comprising a therapeutically effective amount of liposomal Gemcitabine and a negatively charged phospholipid.

2. The method of claim 1, wherein the composition further comprises a pharmaceutically acceptable excipient.

3. The method of claim 1 or 2, wherein the Gemcitabine is gemcitabine hydrochloride, gemcitabine free base, a gemcitabine derivative, or a mixture thereof.

4. The method of any of claims 1-3, wherein said negatively charged lipid comprises a cardiolipin selected from the group consisting of natural cardiolipin and synthetic cardiolipin.

5. The method any of claims 1-4, wherein said liposome bears a negative charge.

6. The method any of claims 1-4, wherein said liposome bears a positive charge

7. The method of any of claims 1-6, wherein at least a portion of said gemcitabine is complexed with negatively charged lipid through electrostatic interaction.

8. The method of any of claims 1-7, wherein said liposomes are a mixture of multilamellar vesicles and unilamellar vesicles.

9. The method of any of claims 1-8, wherein said pharmaceutical composition further comprises one or more therapeutic agents other than gemcitabine.

10. The method of claim 9, wherein one or more of said agents is an antineoplastic, antifungal, or antibiotic agent.

11. A liposomal composition comprising gemcitabine, a first liposome forming material comprising cardiolipin, and a second liposome forming material.

12. The composition of claim 11, wherein the Gemcitabine is gemcitabine hydrochloride, gemcitabine free base, a gemcitabine derivative, or a mixture thereof.

13. The composition of claim 11 or 12, wherein a portion of said cardiolipin is complexed with said gemcitabine.

14. The composition of any of claims 11-13, wherein said liposome entrapped gemcitabine comprises vesicles having a size of about 5 μm or less.

15. The composition of any of claims 11-13, wherein said liposome entrapped gemcitabine comprises vesicles having a size of about 1 μm or less.

16. The composition of any of claims 11-13, wherein said liposome entrapped gemcitabine comprises vesicles having a size of about 0.5 μm or less.

17. The composition of any of claims 11-13, wherein said liposome entrapped gemcitabine comprises vesicles having a size of about 0.1 μm or less.

18. The composition of any of claims 11-17, wherein said second liposome-forming material is a lipid selected from the group consisting of phosphatidylcholine, cholesterol, α-tocopherol, phosphatidylglycerol and phosphatidyl serine.

19. The composition of any of claims 11-18, wherein said cardiolipin is selected from the group consisting of natural cardiolipin and synthetic cardiolipin.

20. The composition of any of claims 11-19, wherein said liposome bears a negative charge.

21. The composition of any of claims 11-19, wherein said liposome bears a positive charge.

22. The composition of any of claims 11-19, wherein said liposome is neutral.

23. The composition of any of claims 11-22, wherein said liposome is a mixture of multilamellar vesicles and unilamellar vesicles.

24. The composition of any of claims 11-23, wherein said pharmaceutical composition further comprises one or more therapeutic agents other than gemcitabine.

25. The composition of claim 24, wherein one or more of said agents is an antineoplastic, antifungal, or antibiotic agent.

26. The composition of claim 24, wherein one or more of said agents is cisplatin, an antisense oligonucleotide, oxaliplatin, paclitaxel, vinorelbine, or epirubicin.

27. The composition of claim 24, wherein one of more of said agents is an oligonucleotide antisense to raf.

28. The composition of any of claims 11-27, further comprising one or more pharmaceutical acceptable excipients.

29. The composition of claim 28, wherein one or more of said excipients enhances shelf-life of the composition.

30. The composition of claim 28, wherein one or more of said excipients improves the stability of the composition.

31. The composition of any of claims 28-30, wherein one or more of said excipients is a sugar

32. The composition of claim 31, wherein the sugar is selected from the group consisting of trehalose, maltose, sucrose, glucose, lactose, and dextran.

33. The composition of claim 31 or 32 wherein the sugar is trehalose.

34. The composition of claim 31 or 32 wherein the sugar is sucrose.

35. The composition of claim 31 wherein the sugar is an aminoglycoside.

36. The composition of claim 35 wherein the aminoglycoside is streptomycin.

37. The composition of claim 35 wherein the aminoglycoside is dihydrostreptomycin.

38. A method for the treatment of a cellular proliferative disease comprising administering a therapeutically effective amount of the composition of any of claims 11-37 a patient in need thereof.

39. The method of any of claims 1-10 or 38, wherein said patient is human.

40. The method of any of claims 1-10 or 38-39, wherein said a cellular proliferative disease is cancer.

41. The method of claim 40, wherein the cancer is lymphoma, ovarian cancer, breast cancer, pancreatic cancer, lung cancer, or colon cancer.

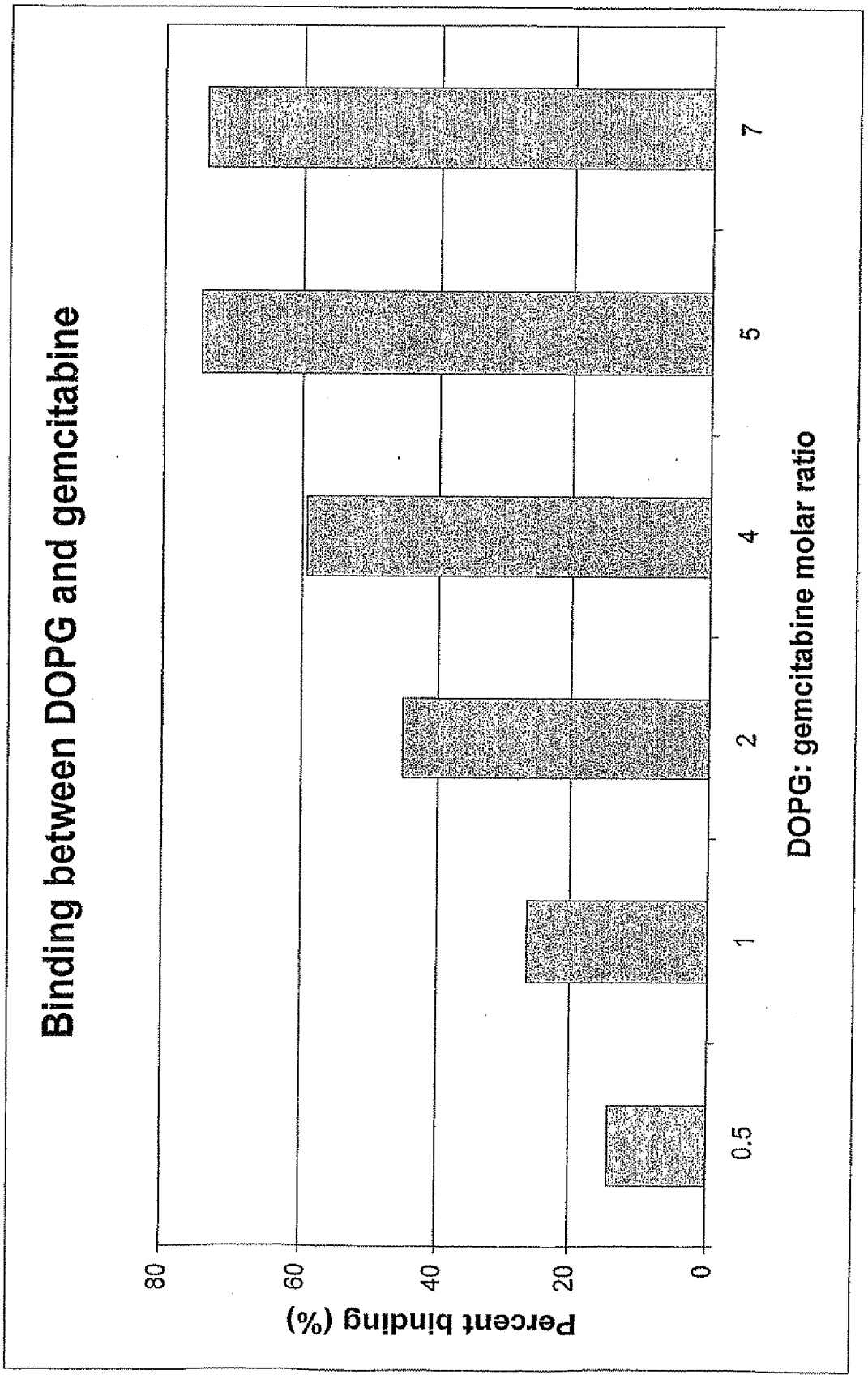
Binding between DOPG and gemcitabine
Percent binding (%)
0
20
40
60
80
0.5
1
2
4
5
7
DOPG: gemcitabine molar ratio

Figure 1

223836.ST25
SEQUENCE LISTING

<110> NeoPharm, Inc.

<120> Gemcitabine Compositions For Better Drug Delivery

<130> 223836

<150> 60/405,378
<151> 2002-08-23

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<170> PatentIn version 3.2

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<212> DNA
<213> Artificial

<220>
<223> Anti-raf-oligonucleotides

<400> 1
gtgctccatt gatgc 15

INTERNATIONAL SEARCH REPORT

Internatic ** lication No
PCT/L. ../25293

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/127 A61K31/7068

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	EP 0 750 910 A (YOSHITOMI PHARMACEUTICAL) 2 January 1997 (1997-01-02) page 3, line 28 -page 5, line 6 examples	1-3,5-10 1-41
X Y	WO 99 49716 A (MOOG REGINA ;UNGER CLEMENS (DE); MASSING ULRICH (DE)) 7 October 1999 (1999-10-07) page 6, line 6 - line 23 page 7, line 10 - line 13	1-3,5-10 1-41
Y	US 4 419 348 A (RAHMAN AQUILUR ET AL) 6 December 1983 (1983-12-06) column 2, line 13 - line 60 examples	1-41

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 December 2003	23/12/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Giménez Miralles, J

INTERNATIONAL SEARCH REPORT

Internatic lication No
PCT/L /25293

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GOKHALE PC ET AL: "AN IMPROVED METHOD OF ENCAPSULATION OF DOXORUBICIN IN LIPOSOMES: PHARMACOLOGICAL, TOXIGOLOGICAL AND THERAPEUTIC EVALUATION" BRITISH JOURNAL OF CANCER, LONDON, GB, vol. 74, no. 1, July 1996 (1996-07), pages 43-48, XP000961424 ISSN: 0007-0920 See "Preparation of liposomes" on p.44	1-41
Y	US 6 146 659 A (RAHMAN AQUILUR) 14 November 2000 (2000-11-14) column 2, line 64 -column 3, line 64 examples	1-41
Y	WO 02 32400 A (AHMAD IMRAN ;NEOPHARM INC (US); RAHMAN AQUILUR (US)) 25 April 2002 (2002-04-25) page 2, line 6 - line 29 examples	1-41
Y	US 4 952 408 A (RAHMAN AQUILUR) 28 August 1990 (1990-08-28) column 2, line 30 -column 3, line 36	1-41
Y	EP 0 286 418 A (US COMMERCE) 12 October 1988 (1988-10-12) page 3, line 25 -page 4, line 20 examples	1-41
Y	US 5 246 708 A (BAMAT MICHAEL K ET AL) 21 September 1993 (1993-09-21) example 6	1-41
Y	US 6 126 965 A (KASID USHA ET AL) 3 October 2000 (2000-10-03) column 1, line 35 - line 46 column 4, line 65 -column 5, line 20	1-41

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-10 and 38-41 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 03/25293

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

on patent family members

Internatic cation No
PCT/I 25293

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
EP 0750910	A	02-01-1997	EP	0750910	A1	02-01-1997
			US	5776488	A	07-07-1998
			CA	2184834	A1	14-09-1995
			WO	9524201	A1	14-09-1995
WO 9949716	A	07-10-1999	DE	19813773	A1	30-09-1999
			WO	9949716	A2	07-10-1999
			EP	1087752	A2	04-04-2001
			JP	2002509866	T	02-04-2002
US 4419348	A	06-12-1983	AT	17319	T	15-01-1986
			AU	552812	B2	19-06-1986
			AU	8520982	A	24-11-1982
			DE	3268367	D1	20-02-1986
			DK	573082	A ,B,	23-12-1982
			EP	0077377	A1	27-04-1983
			JP	5026767	B	19-04-1993
			JP	58500715	T	06-05-1983
			WO	8203769	A1	11-11-1982
US 6146659	A	14-11-2000	AU	730599	B2	08-03-2001
			AU	5088099	A	24-01-2000
			BR	9906581	A	26-09-2000
			CA	2301057	A1	13-01-2000
			CN	1275076	T	29-11-2000
			EA	3678	B1	28-08-2003
			EP	1009384	A1	21-06-2000
			HU	0004583	A2	28-08-2001
			JP	2001524990	T	04-12-2001
			NO	20001012	A	15-03-2000
			PL	338951	A1	04-12-2000
			SK	4642000	A3	07-11-2000
			TR	200000576	T1	21-11-2000
			WO	0001366	A1	13-01-2000
			US	2003035830	A1	20-02-2003
WO 0232400	A	25-04-2002	AU	1464902	A	29-04-2002
			CA	2424345	A1	25-04-2002
			EP	1333811	A1	13-08-2003
			NO	20031623	A	05-06-2003
			WO	0232400	A1	25-04-2002
			US	2003219476	A1	27-11-2003
US 4952408	A	28-08-1990	AT	103172	T	15-04-1994
			AU	625308	B2	09-07-1992
			AU	3742489	A	12-12-1989
			CA	1339077	C	29-07-1997
			DE	68914154	D1	28-04-1994
			DE	68914154	T2	20-10-1994
			DK	279890	A	23-11-1990
			EP	0416014	A1	13-03-1991
			IE	64513	B1	09-08-1995
			JP	3504381	T	26-09-1991
			WO	8911292	A1	30-11-1989
EP 0286418	A	12-10-1988	AT	77945	T	15-07-1992
			AU	601848	B2	20-09-1990
			AU	1701788	A	04-11-1988

INTERNATIONAL SEARCH REPORT

on patent family members

Internati — ication No
PCT/US 03/25293

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0286418 A		CA 1315684 C	06-04-1993
		DE 3872563 D1	13-08-1992
		DE 3872563 T2	03-12-1992
		EP 0286418 A1	12-10-1988
		IL 86009 A	16-09-1991
		JP 2501576 T	31-05-1990
		WO 8807854 A1	20-10-1988
US 5246708 A	21-09-1993	US 5770582 A	23-06-1998
		AT 142221 T	15-09-1996
		AT 243039 T	15-07-2003
		AU 2722688 A	23-05-1989
		AU 610315 B2	16-05-1991
		CA 1329932 C	31-05-1994
		DE 3855513 D1	10-10-1996
		DE 3855513 T2	09-01-1997
		DE 3856557 D1	24-07-2003
		DK 318089 A	28-08-1989
		EP 0355131 A1	28-02-1990
		EP 0712629 A1	22-05-1996
		FI 893100 A ,B,	26-06-1989
		FI 941245 A ,B,	16-03-1994
		IL 88204 A	18-06-1996
		IN 167609 A1	24-11-1990
		JP 2764014 B2	11-06-1998
		JP 7267981 A	17-10-1995
		JP 2500373 T	08-02-1990
		JP 2637534 B2	06-08-1997
		KR 9207395 B1	31-08-1992
		KR 9303495 B1	01-05-1993
		NO 892642 A ,B,	24-08-1989
		WO 8903838 A1	05-05-1989
		US 6020320 A	01-02-2000
		US 5736531 A	07-04-1998
		US 2001025032 A1	27-09-2001
		US 6054441 A	25-04-2000
		US 6060459 A	09-05-2000
		US 6297222 B1	02-10-2001
		US 6103701 A	15-08-2000
		US 5968914 A	19-10-1999
		US 6348451 B1	19-02-2002
		US 6306834 B1	23-10-2001
		ZA 8808083 A	27-06-1990
		AT 97574 T	15-12-1993
		AU 621368 B2	12-03-1992
		AU 3571389 A	24-11-1989
		DE 68910973 D1	05-01-1994
		DE 68910973 T2	17-03-1994
		DK 256290 A	21-12-1990
		EP 0420862 A1	10-04-1991
		HK 1005703 A1	22-01-1999
		IN 169469 A1	19-10-1991
		JP 6035389 B	11-05-1994
		JP 3502694 T	20-06-1991
		MX 171542 B	04-11-1993
		NO 904611 A	05-12-1990
		RU 2115410 C1	20-07-1998
		WO 8910129 A1	02-11-1989

INTERNATIONAL SEARCH REPORT

Internatio lication No
PCT/US 03/25293

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6126965 A	03-10-2000	AU 749927 B2	04-07-2002
		AU 8938098 A	20-10-1998
		EP 0983513 A1	08-03-2000
		US 2003215489 A1	20-11-2003
		WO 9843095 A1	01-10-1998
		US 6333314 B1	25-12-2001
		US 6559129 B1	06-05-2003
		US 2002160038 A1	31-10-2002